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Capture of Particles by Direct Interception by Cilia During Feeding of a Gastropod Veliger

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Abstract. Ciliary feeders vary in the arrangement of ciliary bands and mechanisms of capture of food. Some larvae use opposed parallel bands of preoral (prototroch) and postoral (metatroch) cilia. Hypotheses for the mechanism of particle capture include filtration by adhesion to a cilium that overtakes a particle (direct interception), but until now unequivocal evidence for this mechanism has been lacking. Here, high-speed video recordings of veliger larvae of the gastropod *Lacuna vincta* demonstrated direct interception of particles by prototrochal cilia. Adhesion between cilium and particle was seen when a prototrochal cilium tugged a diatom chain into the food groove while in contact with one part of the chain. In several recorded events, a prototochal cilium overtook a particle during its effective stroke and subsequently pulled the particle inward with its recovery stroke; thereupon, the particle was deposited onto the food groove and transported to the mouth. Captures varied, however. In some cases the particle was intercepted multiple times in one capture event; in others, several cilia passed a particle without interception. Particles occasionally remained in the area of recovery strokes, indicating retention without continuing adhesion to a cilium. In three events, a particle lost from prototrochal cilia was intercepted and moved into the food groove by metatrochal cilia. Particles as wide as or wider than the food groove were also captured and transported but were not ingested.

Introduction

Several distinct modes of ciliary capture and ingestion are widespread among marine larvae across phyla. Researchers have categorized them according to form and supposed function (Riisgård *et al.*, 2000). One common arrangement of cilia is circumferential opposed preoral and postoral bands of cilia (prototroch and metatroch) bordering a ciliated food groove (Fig. 1). The prototrochal cilia produce a current for feeding and swimming with effective strokes in an arc from anterior to posterior. The effective strokes of the metatrochal cilia beat in an arc from posterior to anterior. They oppose the prototrochal cilia but are much shorter. Captured particles are conveyed along the food groove to the mouth. This feeding apparatus is found in mollusc veliger larvae, some annelid larvae, and some rotifers (Strathmann *et al.*, 1972; Riisgård *et al.*, 2000). This opposed-band feeding mechanism is described from some entoproct larvae (Jägersten, 1964), although recent observations have cast doubt on that interpretation for another entoproct larva (Haszprunar and Wanninger, 2008). In an apparently similar kind of ciliary feeding by sessile animals (sabellid and serpulid annelids, entroprocts), the opposed bands are composed of cilia of equal length (Henderson and Strathmann, 2000; Riisgård *et al.*, 2000).

Particle capture has been studied more extensively with mollusc veligers than with other opposed-band feeders, but the mechanism by which the velar cilia capture particles is not clear. Some observations suggest that the prototrochal cilia capture particles by direct interception (Strathmann *et al.*, 1972; Strathmann and Leise, 1979; Riisgård *et al.*, 2000; Strathmann and Grünbaum, 2006). Direct interception requires motion of a structure (in this case a cilium) relative to a particle and adhesion between the structure and particle once contact is made. The ratio of velocities of prototroch cilia to nearby particles is usually above 1 (i.e., the cilium moves faster than the particle: Strathmann and Leise, 1979; Emlet, 1990), indicating that direct interception of particles is possible if a particle adheres to a cilium (LaBarbera,

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Abbreviations: C/P, ratio of cilium velocity to particle velocity; FSW, filtered seawater; FVE, from velar edge.

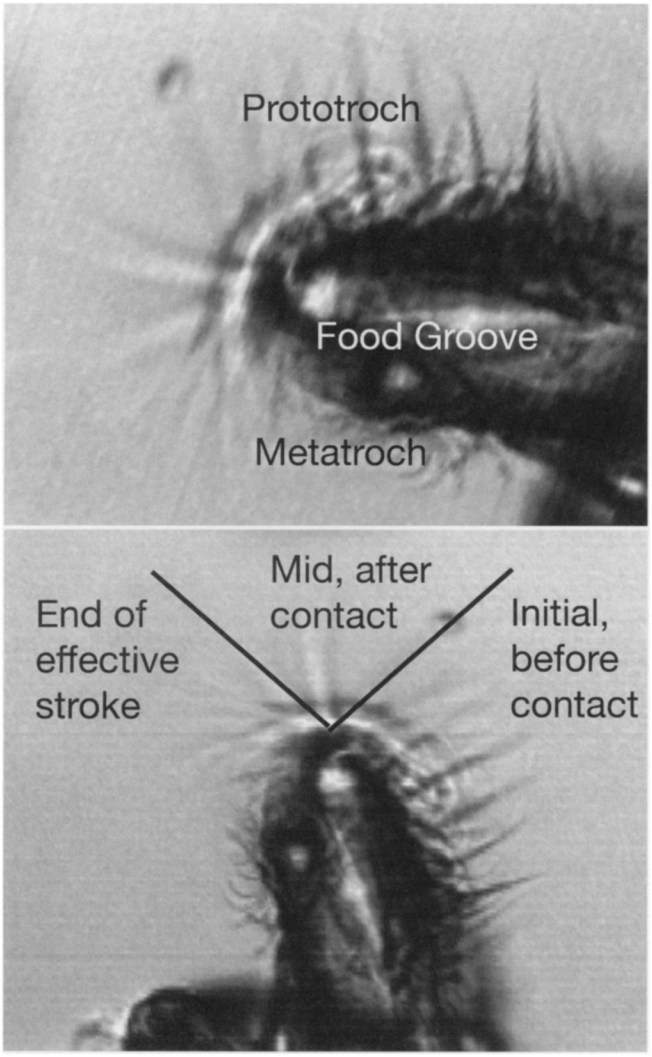

Figure 1. Top: Opposed prototrochal and metatrochal ciliary bands and food groove in a gastropod veliger (*Lacuna vincta*). Bottom: Three segments of the prototroch effective stroke: Initial—before initial particle interception; Mid—near the middle of the effective stroke, after particle interception; End—last third of effective stroke either before the particle was transferred to the food groove or before the particle was lost.

1984; Shimeta and Jumars, 1991). However, direct evidence is lacking, and other processes may contribute to capture of particles (Gallager, 1988). The recovery strokes of the prototroch are involved in captures in ways that have been variously interpreted, and the opposed beat of the metatrochal cilia may also aid captures (Strathmann *et al.*, 1972; Strathmann and Leise, 1979; Gallager, 1988; Riisgård *et al.*, 2000; Strathmann and Grünbaum, 2006).

Adhesion between cilia and particles is also unproven. Lebour (1931) said that mucus appears to be secreted in the food groove of a gastropod veliger but did not investigate its exact location. If adhesion of particles to cilia is important in the capture of food, then secretions affecting adhesion might regulate clearance rates. It has been shown for a bivalved veliger that the capture and transfer of particles is not linked to the palatability of particles but instead is a function of particle encounter rate, suggesting that the mouth is the first place of assessment of incoming particles (Gallager, 1988). In contrast, Fretter and Montgomery (1968) describe regulation of uptake of food at both the velum and the mouth but do not describe how capture rates at the velum are regulated.

Direct interception is qualitatively consistent with the size of particles captured. Particles much smaller than the metachronal wavelength are captured (Strathmann and Leise, 1979; Gallager, 1988; Hansen, 1991, 1993; Riisgård *et al.*, 2000), which would not occur by sieving if the spacing of prototrochal cilia in their effective strokes formed a sieve with mesh size equal to the metachronal wavelength of the ciliary beat. How or whether the mechanism of capture sets the upper limit on particle sizes is unclear, however. The lengths of prototrochal cilia or width of the food groove could possibly set an upper limit to size of particles captured and transported, but reported clearance rates over a range of particle sizes indicate that upper size limits on particles cleared from suspension are less than prototrochal cilium length or width of the food groove (Hansen, 1991, 1993; Riisgård *et al.*, 2000).

This study aimed to (1) acquire a better resolution of the interaction of cilia with particles to test the hypothesis that capture is by direct interception and (2) discover the upper limit on size of particles captured by opposed bands and transported to the mouth. High-speed video demonstrated that prototroch and metatroch cilia of a gastropod veliger, *Lacuna vincta*, captured particles by overtaking and intercepting them and that the velar cilia caught and transported particles larger than the food groove, though these were not ingested.

Materials and Methods

Collection of egg masses and larval culture

Egg masses of *Lacuna vincta* (Montagu, 1803) were collected from eelgrass at Cattle Pt., San Juan Island, Washington, on 16 July 2007 and again the following summer on 17 July 2008. Egg masses were kept in the laboratory in 10-μm-filtered seawater (FSW) at between 11 °C and 13 °C until hatching. Larvae used in 2007 hatched on 17 and 19 July and in 2008 hatched on 24 July; both batches of larvae hatched with a mean greatest length of shell of 200 μm (n = 5 for each batch). Upon hatching, all larvae were transferred to small culture bowls with FSW and fed a phytoplankton diet of *Isochrysis galbana*. Phytoplankton and FSW were replaced daily, and dead or heavily fouled veligers were removed from cultures.

Observational setup and high-speed video recording

Larvae were given no food for a minimum of 4 h and a maximum of 24 h before observation. Individual veligers

were placed in new 4-ml plastic petri dishes with a large drop of FSW. The fouling on their shells allowed them to adhere to the bottom of the dish and become tethered for observation. This tethering method, as opposed to tethering larvae between a slide and cover glass slip, decreased effects of walls on larval behavior and on water currents around a larva. Temperatures during observations ranged from 18 to 21 °C.

We used three types of phytoplankton—*Isochrysis galbana* (cell diameter ≈5 μm), *Skeletonema costatum* (cell diameter ≈5 μm, in chains up to 16 cells), and *Rhodomonas* sp. (cell diameter ≈12 μm)—and four size categories of polystyrene divinylbenzene plastic spheres—2 μm, 20 μm, 30 μm, and 40 μm—for feeding observations. High concentrations of particles ensured frequent captures within view. Plastic spheres sank and frequent pipetting was required to suspend the spheres in the water. (The spheres are supplied for calibration of particle counters, and their specific gravity is reported as about 1.05.)

Cilium movement and feeding events were observed with a compound microscope with DIC optics under 10× or 20× objective lens magnification. Events were recorded *via* high-speed video with a Redlake Motionscope video camera. Recordings were captured at 1× shutter speed at 250 or 500 frames per second (fps) and played back at 30 or 60 fps into Apple iMovie 6.0.3 for analysis. The intervals between images were more frequent than in previous studies so that relative motion of a particle and a nearby cilium could be tracked during the cilium's effective stroke. At 500 fps, intervals between frames were 0.002 s and resolution 320 by 280 pixels.

Analysis of video clips

Clips at 500 fps were traced on transparencies frame by frame for analysis of cilium and particle paths. Measurements were from 7 video clips from one individual in 2007 and 11 from two individuals in 2008 with the desired velum orientation and with cilium and particle near the focal plane. The larvae in these video clips were 10–11 days posthatching and had a maximum shell length of 280 μm and a mean prototrochal cilium length of 60.5 μm ($n = 5$) for the 2007 individual and 63.5 μm ($n = 5$ each) for the 2008 individuals. Particle paths were observed usually in a ventral view of the velum and sometimes in a dorsal view, with the cilia parallel to the plane of focus. Measurements included distance of particle from velar edge (FVE) and duration of capture event. Cilium (C) and particle (P) velocities were calculated from straight-line segment measurements from cilium to cilium and particle to particle for consecutive frames. Straight-line segments were added and then divided by time. Because arcs were short (less than a 40° angle), straight-line approximations in the 2-ms intervals between frames were within 2% of the true distance.

Velocities of cilia in their effective strokes increase with distance from the base of the cilium. Angular velocities are therefore expected to vary less than velocities at different distances along a cilium. In our data, cilium velocities were greater toward the cilium tips, as expected, but there was much variation, presumably from differences in velocities of strokes and errors in estimating position of the base of a cilium at the velar edge. We therefore compared ratios of velocities of cilium and particle and did not estimate angular velocities.

Cilium and particle velocities were obtained for direct captures for three segments of the prototroch effective stroke: (1) before initial particle interception, (2) from interception through the middle of the effective stroke, and (3) at the end of the effective stroke, which was in the last third of the stroke, before the particle was transferred to the food groove (Fig. 1). Because particles were intercepted at different parts of effective strokes, the boundary between initial and mid stroke, at which initial particle interception occurred, differed among captures, but interceptions were usual by the middle third of the effective stroke. For non-capture events (particle missed), cilium and particle velocities were obtained for the middle third of the effective stroke.

Results

Direct interception of particles by prototrochal cilia

Video recordings confirmed key elements of the hypothesis of capture by direct interception by cilia in their effective strokes (the catch-up hypothesis). In this hypothesis a cilium captures a particle by adhering to it. To contact the particle, a prototrochal cilium overtakes a particle during its effective stroke. With contact and adhesion, the prototrochal cilium accelerates the particle, and cilium and particle then travel together through the rest of the cilium's effective stroke. The captured particle is predicted to travel in a curved path with the ciliary effective stroke and with a cilium -to-particle velocity ratio (C/P) of 1 or close to 1. An extension of this hypothesis is that the cilium takes the particle inward with its recovery stroke toward the lateral edge of the velum, where the particle is subsequently moved into the food groove. If the cilium does not adhere to the particle, it then passes the particle and there is no capture by direct interception.

Direct interception requires adhesion of cilium and particle. Two captures of chains (6 cells long) of the diatom *Skeletonema costatum* demonstrated adhesion (Fig. 2). A prototrochal cilium contacted part of the chain of cells and then pulled the chain in toward the food groove despite the drag on the chain (Fig. 2D, E and G–I). In these observations, the capturing cilium was in its straight effective stroke and then began its recovery stroke while in contact with the particle (see video clips #243 and #244, http://www.biolbull.org/supplemental/).

Interceptions occurred along the length of the cilium,

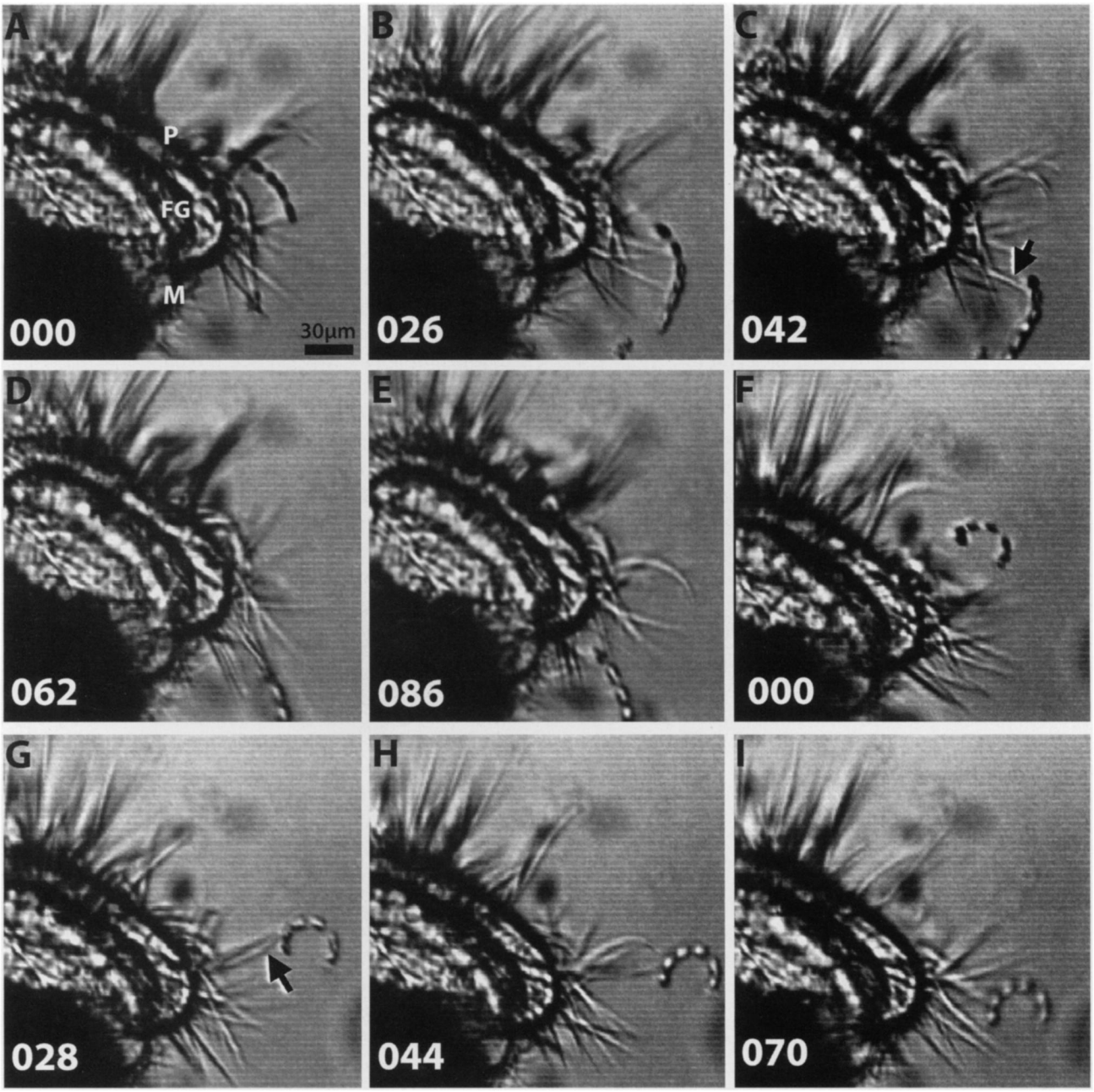

Figure 2. Adhesion between cilium and cells of the diatom *Skeletonema costatum* (see supplementary video clips #243 and #244, http://www.biolbull.org/supplemental/). Dorsal view of velar lobe, with prototroch (P), metatroch (M), and food groove (FG) indicated in frame A. Numbers at lower left are times in milliseconds. (A) Diatom chain is within the zone swept by the prototroch's effective stroke. (B) At least three cilia are associated with the chain. (C) One cilium (black arrow) in contact with one part of the chain. (D, E) Cilium tugs diatom chain toward the food groove. (F) Same individual moments later, with a second diatom chain free of cilia. (G) One part of chain in contact with cilium (black arrow) and (H, I) tugged toward food groove as cilium bends.

with the farthest at 62.5 μm and the closest at 17.5 μm FVE (Fig. 3)—presumably the distance from the base of prototrochal cilia. Particles were also missed along the length of the cilium, with the farthest at 52.5 μm FVE and the closest at 17.5 μm FVE (Fig. 3). Cilium and particle velocity and C/P velocity ratio for captures by direct interception of *Isochrysis galbana*, *Rhodomonas* sp., *Skeletonema costatum*, and a 2-μm plastic sphere are summarized in Table 1 for initial, middle, and end of effective stroke. (These captures are included in Fig. 3.) The C/P ratio was greater near the beginning of the effective stroke and closer to 1 at the middle and end of the effective stroke ($n = 4$ captures). For all particle types the particle accelerated from initial to mid effective stroke ($n = 8$ captures, $P < 0.05$, sign test). This

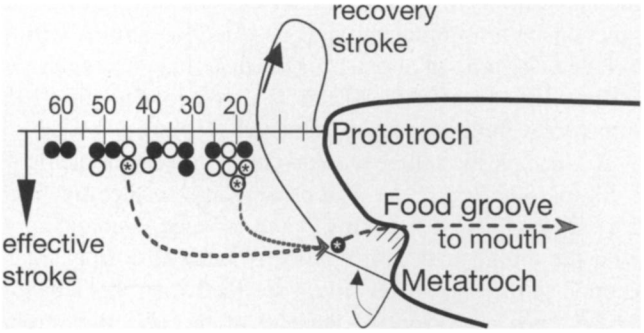

Figure 3. Particle capture success from direct interception along length of prototroch (P) cilia. Units are in micrometers. The prototroch cilia are almost straight in their effective strokes and move in an arc from anterior to posterior. The cilia are bent during the recovery strokes. Successful captures (●) culminated in transfers of particles to the food groove, and misses (○) culminated in loss of particles. Three particles (*) were lost from a prototroch cilium and then captured by a metatroch cilium (see example in Fig. 11). Arrows show directions of movement of cilia.

acceleration of a particle corresponded to the cilium having overtaken the particle and then moving with it. The greatest cilium and particle velocities (5.94 and 5.63 μm ms^{-1}, respectively) at the mid effective stroke occurred during an interception at the tip of the cilium (62.5 μm FVE). A general trend was greater cilium and particle velocities farther from the velar edge (Fig. 4A, B). Cilium velocities were similar in both captures and non-captures, and particle velocities were greater in captures than in non-captures (Fig. 4A, B). Ratios of cilium velocity to particle velocity (C/P) in the capture events were closer to 1 than in the non-capture events (Fig. 4C), as predicted for direct interception. Several examples of these captures follow.

In a direct interception capture of an *I. galbana* cell (Fig. 5; also see video clip #154, http://www.biolbull.org/supplemental/), the particle entered the zone swept by prototrochal cilia at 52.5 μm FVE with a velocity of 3.13 μm ms^{-1} (Fig. 5A). The particle slowed to 2.75 μm ms^{-1} with a C/P velocity ratio of 1.36 before it was overtaken by a cilium at 42.5 μm FVE (Fig. 5B) and accelerated to 3.75 μm ms^{-1} with a C/P velocity ratio of 1. Both particle and cilium slowed to 2.5 μm ms^{-1} and then to 1.88 μm ms^{-1}, the particle reaching its lowest velocity of 0.83 μm ms^{-1} as it moved with the cilium's recovery stroke inward toward the edge of the velum (Fig. 5E, F). The C/P velocity ratio was 1.18 toward the end of the cilium's effective stroke. The particle remained in the same position for about 12 ms (Fig. 5G) before it moved toward the food groove (Fig. 5H). It continued to move along the food groove with a velocity of 0.34 μm ms^{-1} (Fig. 5I). During the particle's travel with the cilium, it followed the curved arc of the cilium's effective stroke (Fig. 6). Another *I. galbana* cell was intercepted near the tip of a cilium (62.5 μm FVE) (Fig. 7; also see video clip #157, http://www.biolbull.org/supplemental/). The particle was accelerated from 3.13 μm ms^{-1} to a maximum velocity of 5.63 μm ms^{-1} with a C/P velocity ratio of 1.06 (Table 1).

Other particles that were captured by direct interception were *Rhodomonas* sp., *S. costatum,* and a 2-μm plastic sphere. In 2007, we never observed cells of *Rhodomonas* sp. being retained by the cilia or ingested, with ingestion assessed by gut color. In 2008, however, larvae fed a mixture of *Rhodomonas* sp. and *I. galbana* captured and ingested both kinds of algae. In a *Rhodomonas* sp. capture (Fig. 8; Table 1), the particle slowed from 2.44 μm ms^{-1} to 1.56 μm ms^{-1} when the cilium passed it with a C/P velocity ratio of 1.8. The particle then began to move through the effective stroke in association with the cilium (Fig. 8A–C). During the effective stroke the cell of *Rhodomonas* sp. moved from the trailing to the leading side of the cilium (Fig. 8A, B), hence the low C/P velocity ratio of 0.8 at mid effective stroke (Table 1). During a 60-ms interval, the

Table 1

Cilium and particle velocities and C/P velocity ratios in direct interception captures

		Initial effective stroke (before interception)			Mid effective stroke (after interception)			End effective stroke		
Particle	Distance FVE (μm)	Cilium velocity (μm/ms)	Particle velocity (μm/ms)	C/P ratio	Cilium velocity (μm/ms)	Particle velocity (μm/ms)	C/P ratio	Cilium velocity (μm/ms)	Particle velocity (μm/ms)	C/P ratio
Isochrysis galbana	25.0	2.66	1.56	1.71	2.91	2.40	1.21	—	1.41	—
I. galbana	42.5	3.75	2.75	1.36	3.75	3.75	1.00	1.97	1.67	1.18
I. galbana	37.5	—	2.50	—	4.06	3.83	1.06	—	2.19	—
I. galbana	62.5	—	3.13	—	5.94	5.63	1.06	—	2.19	—
Skeletonema costatum	47.5	—	2.50	—	2.50	3.05	0.82	1.88	1.88	1.00
S. costatum	57.5	2.81	2.50	1.12	2.81	2.81	1.00	—	1.56	—
Rhodomonas	27.5	2.81	1.56	1.80	2.50	3.13	0.80	—	0.98	—
2-μm sphere	52.5	—	2.81	—	4.50	4.50	1.00	2.55	2.55	1.00

FVE—From Velar Edge.

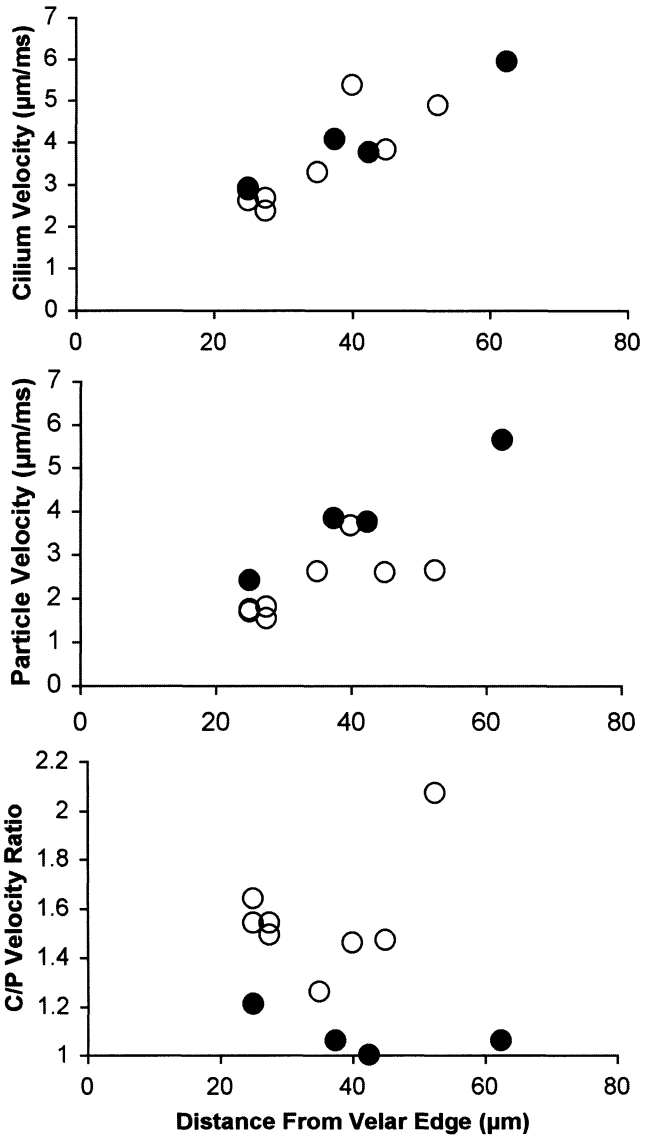

Figure 4. Velocities of *Isochrysis galbana* particles for (●) successful direct interception captures (n = 4) and (○) non-captures (n = 8). (Top) Velocities in mid effective stroke for cilia associated with particles in captures and for cilia passing particles in non-captures. (Middle) Particle velocities in mid effective stroke. (Bottom) Cilium-to-particle velocity ratios (C/P) in mid effective stroke.

particle then moved inward toward the velar edge with the cilium's recovery stroke, where it was rotated by another cilium (Fig. 8D). The movement of the *Rhodomonas* cell to the leading edge of the cilium (Fig. 8A, B) may have resulted from its flagellar beat (see video clip #176, http://www.biolbull.org/supplemental/). In another event, a *Rhodomonas* cell that had been captured and taken along the food groove then reversed along and out of the food groove on a path different from its path of entry, indicating a possible escape by the motile cell.

Two chains of *S. costatum* were overtaken near the tips of prototrochal cilia (at 57.5 and 47.5 μm FVE) with only 148 ms between captures. Each chain of cells traveled in conjunction with a cilium through its effective stroke, with a C/P velocity ratio of about 1 for the first chain and a ratio of 0.82 for the second chain (Table 1; also see video clip #191, http://www.biolbull.org/supplemental/).

A 2-μm plastic sphere was also intercepted near the tip of a cilium (52.5 μm FVE). The particle was accelerated from 2.81 μm ms^{-1} to 4.5 μm ms^{-1} and traveled in conjunction with the cilium with a C/P velocity ratio of 1 from interception to the end of the cilium's effective stroke, slowing to 2.55 μm ms^{-1} toward the end of the effective stroke (Table 1).

Direct interception capture events lasted an average of 159 ms (±29 SD, n = 9) from the time the particle entered the zone swept by prototrochal effective strokes to the time it entered the food groove. Durations of captures were longer than the duration of the 80-ms metachronal waves because of the duration of transfers into the food groove. The average time that particles traveled in conjunction with cilia through the effective stroke was 44 ms (±9 SD, n = 8) and 28 ms (±8 SD, n = 8) on the recovery stroke.

Other captures

Other captures differed from direct interception by a single prototrochal cilium in its effective stroke. In two cases a particle, *I. galbana*, was intercepted by multiple cilia as it passed through the prototrochal effective strokes. These captures appeared to be instances of direct interception but involved more than a single prototrochal cilium. In one of these events the particle was retained within the area of prototrochal recovery strokes for several milliseconds (Fig. 9). In the other event the particle was taken inward with the prototrochal recovery strokes, following a path similar to those in captures with direct interception. In both events the particle passed through the prototrochal effective strokes close to the recovery strokes, 20–15 μm FVE (Fig. 9) and 17.5 μm FVE. One particle (Fig. 9) was intercepted by a total of three cilia, the first of which overtook the particle (Fig. 9B) and accelerated it from 1.25 μm ms^{-1} to 1.88 μm ms^{-1} as it moved through the effective stroke with a C/P velocity ratio of 1 before the particle was "handed-off" to the second cilium (Fig. 9C) at 15 μm FVE. The second cilium and particle had a velocity of 0.94 μm ms^{-1} and a C/P velocity ratio of 1 for the first 8 ms of their 24-ms association. The cilium lost its association with the particle, and a clear association with the third cilium is shown in Figure 9E, 2 ms later. The particle followed the third cilium inward with the cilium's recovery stroke, but instead of moving directly into the food groove the particle was detained within the prototroch's recovery stroke area for 172 ms (Fig. 9F) before it moved toward the food groove (Fig. 9G). A second particle entered the zone swept by prototrochal cilia at 55 μm FVE with a speed of 1.6 μm ms^{-1} as the first particle moved into the food groove (Fig. 9G). The

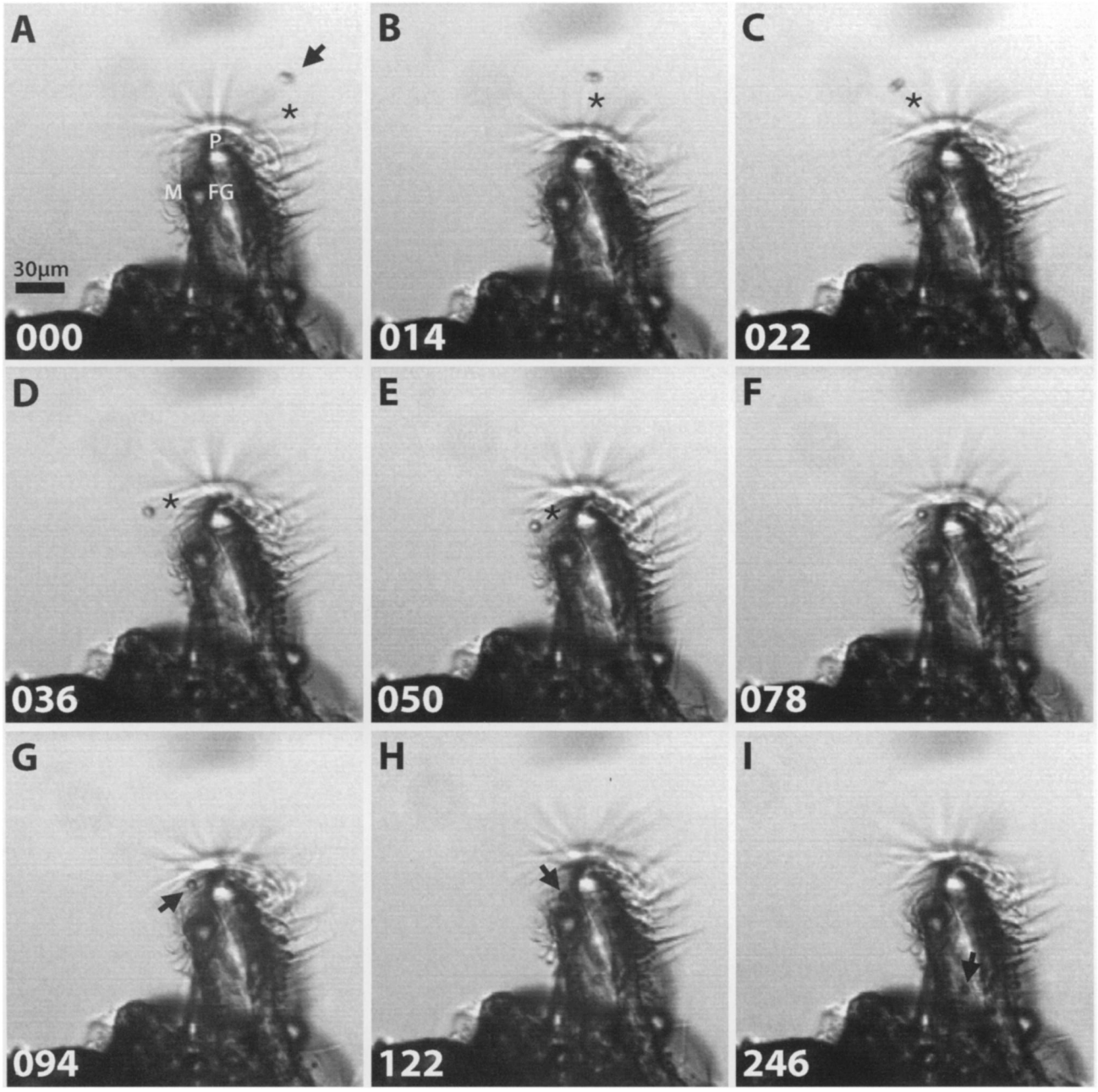

Figure 5. Direct interception of the alga *Isochrysis galbana* (see supplementary video clip #154, http://www.biolbull.org/supplemental/). Ventral-lateral view of velum. Ciliary beat is parallel to the plane of focus. Labels of ciliary bands in frame A and times as in Fig. 2. Symbol * marks cilium in its effective stroke moving initially from anterior to posterior. (A) Particle (black arrow) is in zone swept by prototroch effective strokes. (B) Particle is overtaken by cilium. (C–E) Particle continues to move in conjunction with cilium. (F) Particle is moved inward toward lateral edge of velum with cilium's recovery stroke. (G–I) Black arrowhead points to particle. (G) Particle has remained near lateral edge of velum for 16 ms before (H) it moves toward food groove and (I) is transported on food groove at 0.34 μm ms^{-1}.

second particle accelerated to 2.19 μm ms^{-1} and moved to 27.5 μm FVE while cilia passed it (Fig. 9H, I). A minimum of eight cilia that were visible passed the particle. One of those cilia passed the particle at a velocity of 2.6 μm ms^{-1} with a C/P velocity ratio of 1.19 for 8 ms and then a C/P velocity ratio of 1.59 for 20 ms. The particle slowed to 1.25 μm ms^{-1} and reached 52.5 μm FVE as it left the zone swept by prototrochal effective strokes, and then continued to slow to 0.53 μm ms^{-1} (see video clip #125, http://www.biolbull.org/supplemental/). In two other events, lost particles had a similar trajectory.

Yet another capture differing from direct interception by

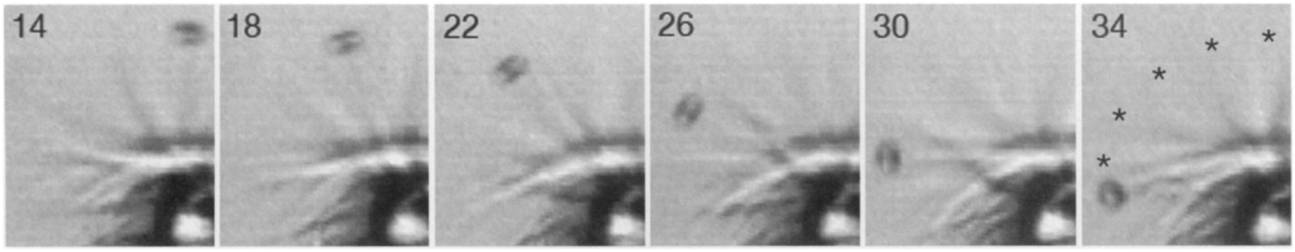

Figure 6. Curved path of particle as the cilium and particle move together toward the food groove during the interception in Fig. 5. Times in milliseconds (upper left) correspond to times in Fig. 5. The positions of the particle in preceding frames are marked in the frame at 34 ms.

a prototrochal cilium occurred when a particle, *I. galbana*, moved through the area swept by the prototrochal effective strokes, remaining close to the recovery strokes (25 μm FVE). Prototrochal cilia in their effective strokes passed the particle without any interceptions (see Fig. 10A–C and video clip #136, http://www.biolbull.org/supplemental/). If the particle was retained by direct interception, it was not until near the end of effective strokes or the beginning of recovery strokes. The particle was detained (as in Fig. 9F, G) in the area of prototrochal recovery strokes (Fig. 10D, E) for 260 ms before leaving the area and moving toward the food groove (Fig. 10F).

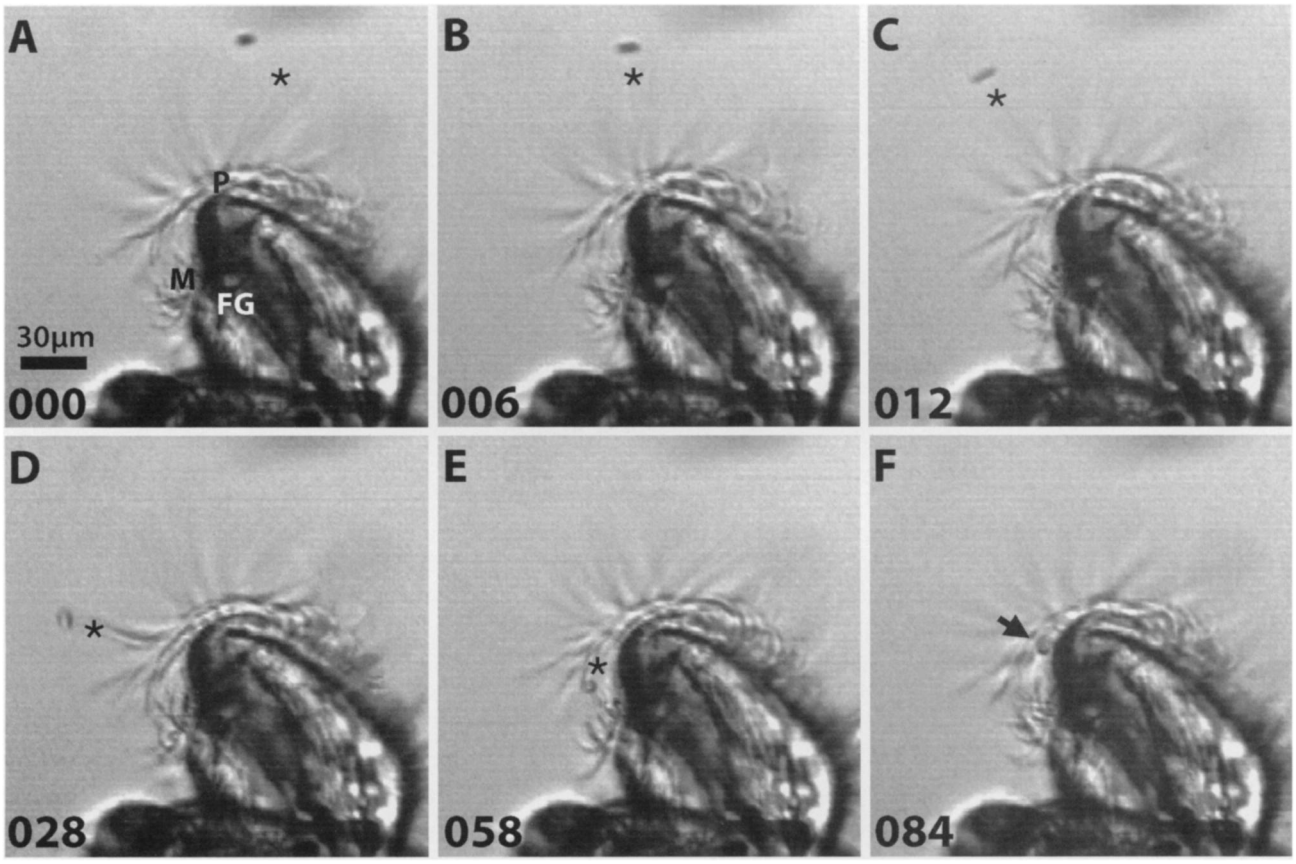

Figure 7. Direct interception of *Isochrysis galbana* at cilium tip (see supplementary video clip #157, http://www.biolbull.org/supplemental/). Ventral-lateral view of velum at a slight angle to the left. Ciliary beat is parallel to the plane of focus. Labels of ciliary bands in frame A and times as in Fig. 2. Symbol * marks cilium in its effective stroke and beginning its recovery stroke. (A) Particle entering zone swept by prototroch effective strokes. (B) Particle is overtaken by tip of cilium and (C) moves in conjunction with cilium. (D) Cilium bends back during its effective stroke as it moves the particle in a curved path. (E) Particle remains at tip of cilium and is taken inward on cilium's recovery stroke to (F) edge of velum (black arrowhead points to particle) before particle moved into the food groove.

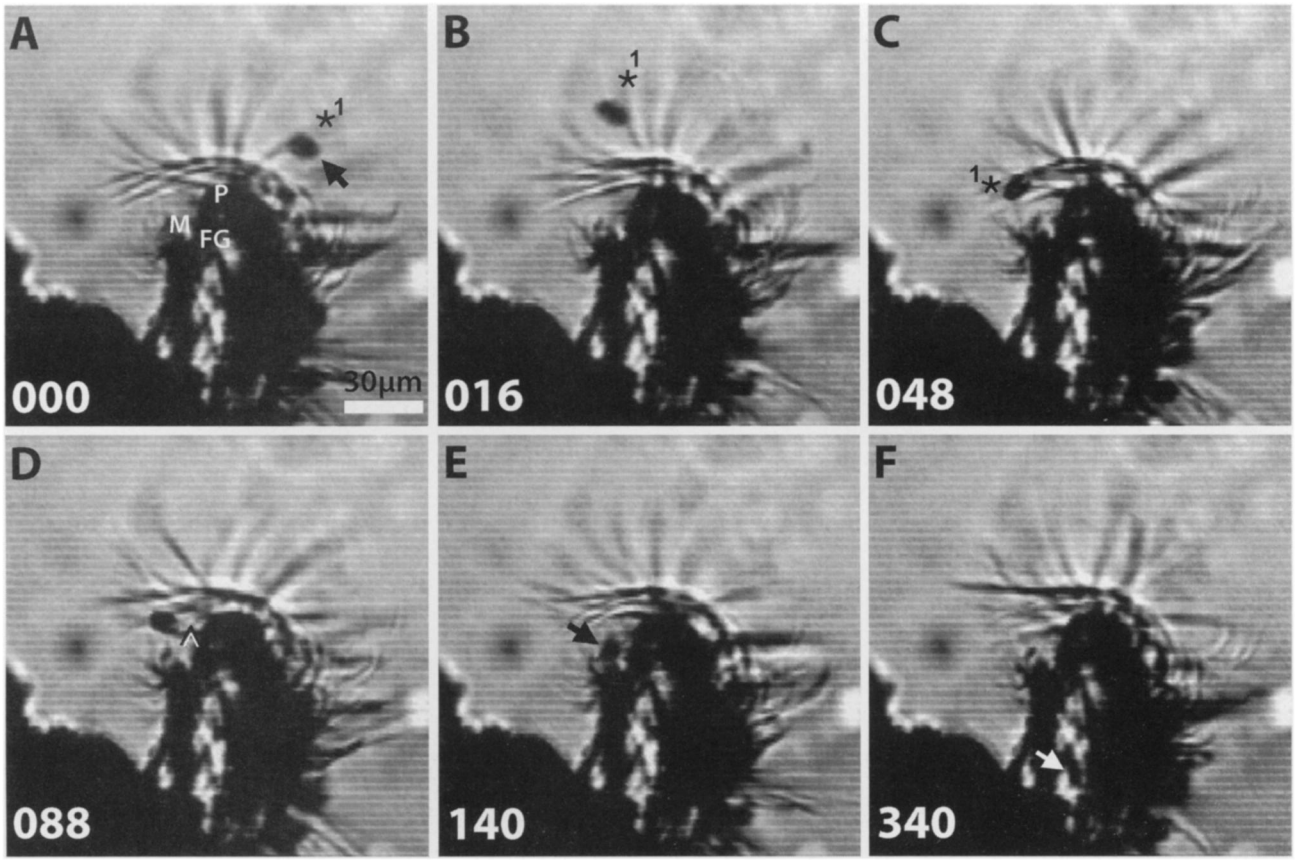

Figure 8. Direct interception of the alga *Rhodomonas* sp. (see supplementary video clip #176, http://www.biolbull.org/supplemental/). Ventral-lateral view of velum. Ciliary beat is parallel to the plane of focus. Labels of ciliary bands in frame A and times as in Fig. 2. Symbol * marks a prototrochal cilium, initially in its effective stroke. (A) Particle joins a cilium as it is being passed by the cilium. (B) Particle continues to move in conjunction with cilium, and in the process moves from the trailing to the leading side of the cilium. (C) Particle remains associated with the cilium and is taken inward with the cilium's recovery stroke. (D) While in the area of recovery strokes the particle is rotated by another cilium marked by ^. (E) The particle (black arrow) moves toward the food groove and (F) travels down the food groove at 0.24 μm ms^{-1} (white arrow points to particle).

Two algal cells, *I. galbana*, and one unidentified 5-μm particle that were lost from prototrochal cilia were then intercepted and moved into the food groove by metatrochal cilia. In two of these events, the particle appeared to be directly intercepted by a prototrochal cilium but lost toward the end of the cilium's effective stroke. During one of these two events (Fig. 11), the unidentified 5-μm particle appeared to be intercepted at 45 μm FVE (Fig. 11B) and slightly accelerated from 2.5 to 2.93 μm ms^{-1} with a C/P velocity ratio of 0.81. It is not likely that the particle in this event was traveling faster than the cilium (see Discussion). Toward the end of the cilium's effective stroke the cilium bent and remained associated with the particle as an adjacent cilium passed (Fig. 11C, D). The particle was lost and free of any cilia for 56 ms (Fig. 11E) before it was overtaken by a metatroch cilium (Fig. 11F, G). The particle remained associated with a metatrochal cilium through its effective stroke and was placed in the food groove (Fig. 11H, I); the capture event lasted a total of 160 ms from the time the particle entered the zone swept by protrochal cilia to the time the particle was placed on the food groove (see video clip #180, http://www.biolbull.org/supplemental/). The velocity of the metatroch cilium with particle was 0.97 μm ms^{-1}, and a different metatroch cilium without a particle had a velocity of 1.25 μm ms^{-1}. In the third event, a particle remained close to the recovery strokes (20 μm FVE) as it moved through the area swept by prototrochal effective strokes without interception and was lost from the prototrochal cilia. The particle was then intercepted repeatedly by metatrochal cilia during the 84 ms before it was placed in the food groove.

Only 612 ms before this event, another particle, *I. galbana*, had been lost as it passed at the tips of the prototrochal cilia. The lost particle had entered the zone swept at 65-μm FVE with a velocity of 1.73 μm ms^{-1} and accelerated to 2.5 μm ms^{-1}. The particle appeared to

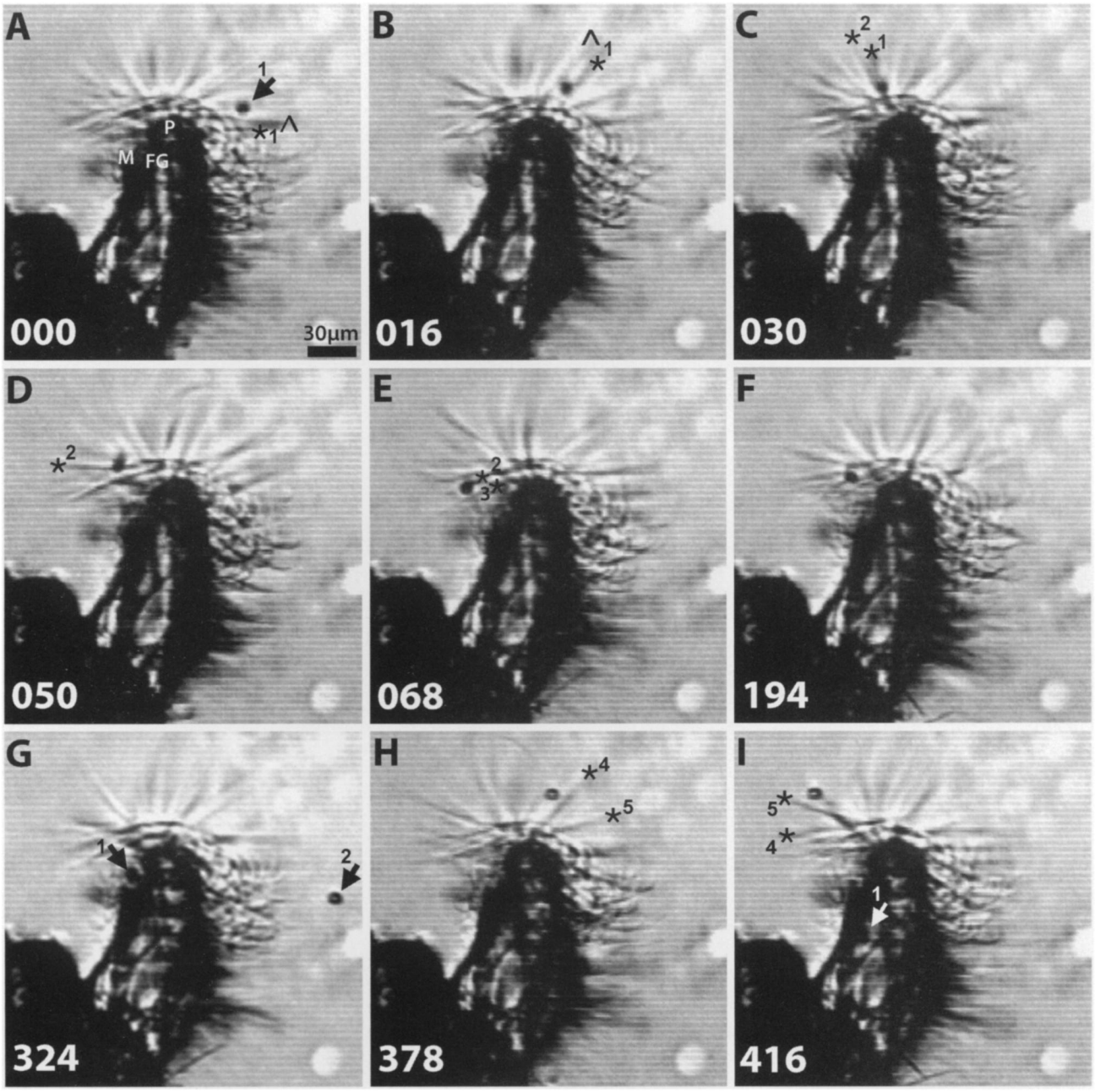

Figure 9. Multiple interceptions in one capture followed by a non-capture of *Isochrysis galbana* particles (see supplementary video clip #125, http://www.biolbull.org/supplemental/). Ventral-lateral view of velum; ciliary beat parallel to the plane of focus. Labels of ciliary bands in frame A and times as in Fig. 2. Symbols ^ and * mark cilia in their effective strokes moving initially from anterior to posterior. Numbered black arrows point to particles. (A) Particle (black arrow 1) close to recovery strokes in zone swept by prototroch effective strokes with two adjacent cilia (^ and *1) behind it. (B) One cilium (^) passes particle and adjacent cilium (*1) overtakes particle. (C) Particle disassociates from first cilium (*1) and appears to associate with a second cilium (*2). (D) Particle moves in conjunction with cilium (*2) to the end of the cilium's effective stroke. (E) As cilium (*2) begins its recovery stroke the particle loses association with it and associates with a third cilium (*3). (F) Particle entrained in prototroch recovery strokes. (G) The first particle (black arrow 1) moves toward food groove as a second particle (black arrow 2) moves toward zone swept by prototrochal cilia. (H, I) Particle is passed by cilia (*4 and *5) as it moves through and later exits the effective stroke without interceptions. White arrow 1 points to first particle on food groove.

be overtaken by a cilium at 50 μm FVE for a mere 4 ms and accelerated to 3.0 μm ms^{-1}, but then the particle immediately slowed to 2.5 μm ms^{-1}, left the zone swept by effective strokes, and continued to slow to 1.5 μm ms^{-1}. Five other recordings of missed particles showed similar trajectories.

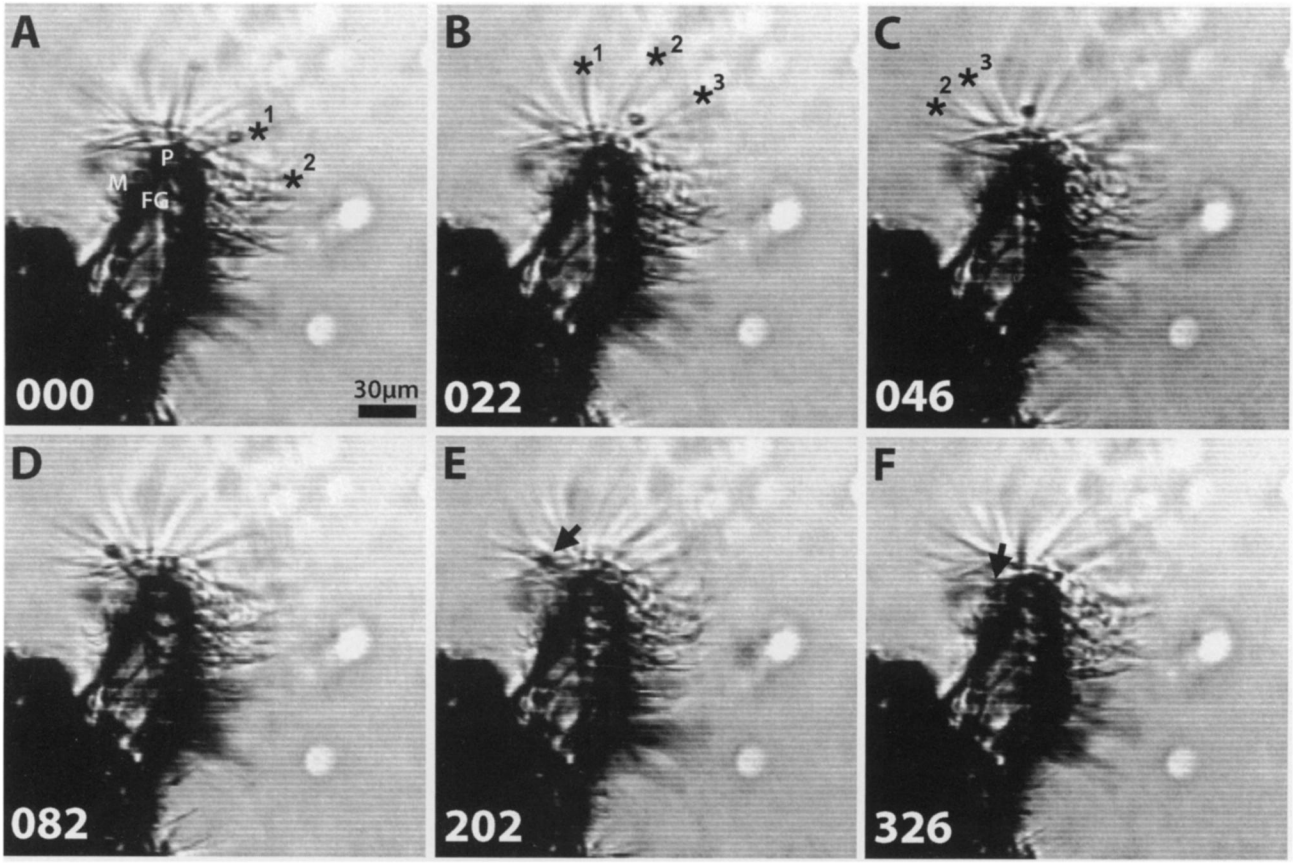

Figure 10. Capture of *Isochrysis galbana* without apparent interception by effective strokes (see supplementary video clip #136, http://www.biolbull.org/supplemental/). Ventral-lateral view of velum; ciliary beat parallel to the plane of focus. Labels of ciliary bands in frame A and times as in Fig. 2. Symbol * marks cilia in their effective strokes moving initially from anterior to posterior. Black arrows point to particle. (A–C) Particle moving across zone swept by prototroch effective strokes close to recovery strokes with cilia *1, *2, and *3 passing the particle. (D) Particle enters area of recovery strokes and (E) is retained within the recovery strokes. (F) Particle moves toward food groove.

Capture of large spheres

A veliger was able to capture and transport 27.5-μm and 44-μm plastic spheres on the food groove (Fig. 12). Large spheres (20–40 μm) were rejected at the mouth; however, veligers did ingest 2-μm spheres, which appeared in their gut. The diameters of the largest captured particles were similar to the metachronal wavelength, 30 μm. At the largest sizes, sieving by prototrochal cilia one metachronal wavelength apart seems a possibility. Adjacent cilia in their effective strokes could form a sieve with mesh size equal to the metachronal wavelength. But the wavelength of 30 μm was too great for sieving the smaller particles (see video clip #298, http://www.biolbull.org/supplemental/).

Discussion

The high-speed video recordings provide strong evidence for capture of particles by direct interception. We observed (1) particles being overtaken by a cilium, accelerated, and then moved in conjunction with the cilium through the remainder of its effective stroke, (2) particles subsequently pulled toward the food groove in the path of a cilium's recovery stroke, (3) a cilium bent and slowed in concomitance with a particle encounter, and (4) a cilium pulling a chain of cells from its contact with one end of the chain. Also, (5) intercepted particles had greater velocities than particles that were missed. In contrast to previous observations of veligers (Strathmann and Leise, 1979; Gallager, 1988), particles were caught along the lengths of the prototrochal cilia nearly to their tips. In addition to observing these events in capture by prototroch cilia, we also witnessed capture near the tip of a metatrochal cilium.

Features of direct interception were apparent in the ratios of cilium-to-particle (C/P) velocities. In captures, C/P velocity ratio was initially greater than 1 (right before interception) and then close to 1 during the middle and end of the effective stroke (after interception) (Table 1). At the middle and end of prototrochal effective strokes, C/P velocity ratio was close to 1 for captured particles and greater for missed particles. Some measured C/P velocity ratios were slightly

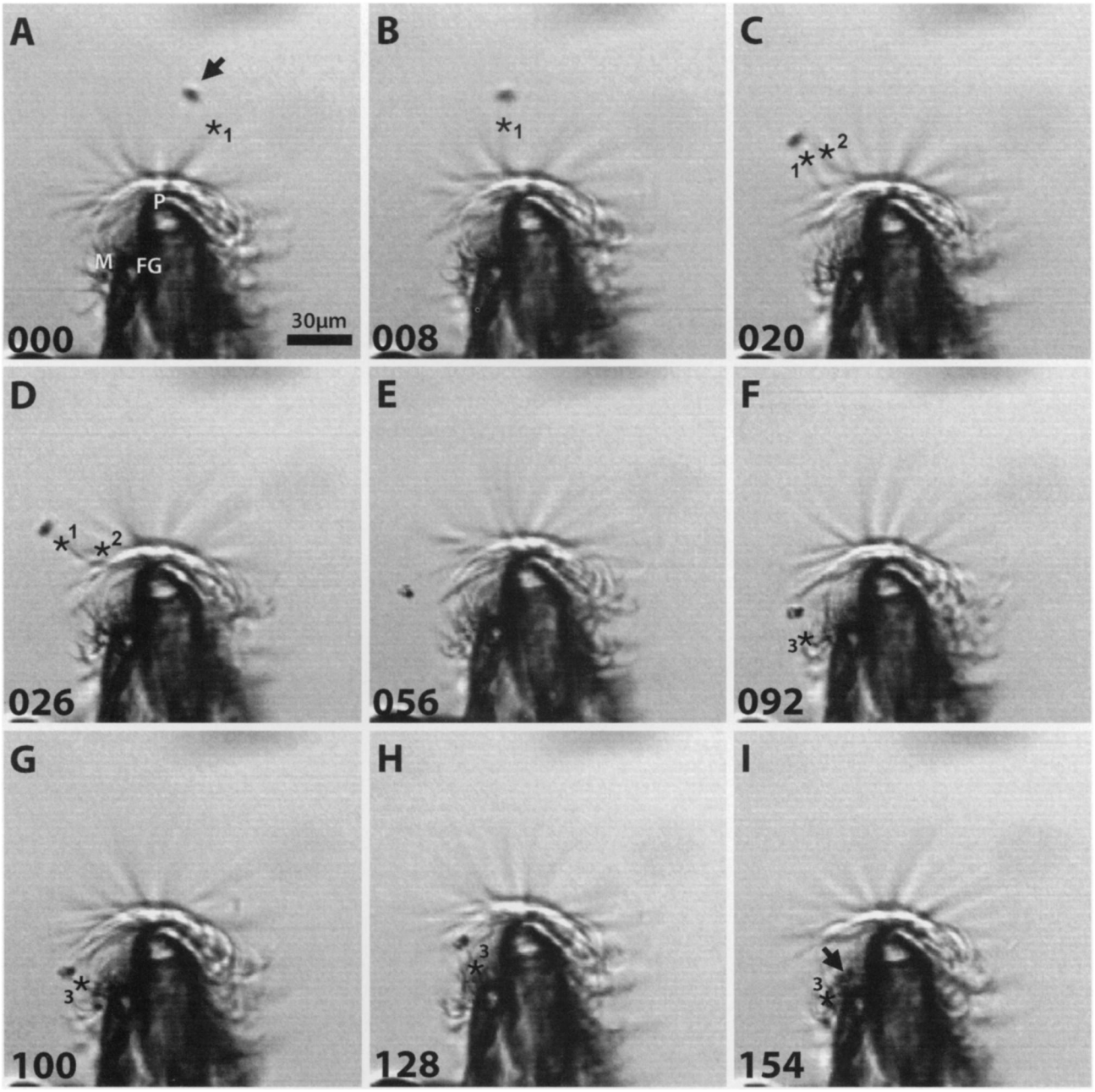

Figure 11. Prototrochal cilium interception and loss followed by a metatrochal interception and capture of an unidentified particle (5 μm in length) (see video clip #180, http://www.biolbull.org/supplemental/). Ventral-lateral view of velum; ciliary beat parallel to the plane of focus. Labels of ciliary bands in frame A and times as in Fig. 2. Symbol * marks cilia in their effective strokes. (A) Particle (black arrow) is in the zone swept by the prototroch's effective strokes. (B) A prototrochal cilium (*1) overtakes particle. (C) The cilium (*1) begins to bend and remains associated with particle (*2 marks adjacent cilium). (D) Cilium (*2) passes particle and cilium (*1), still in association. (E) Particle is lost and distant from prototroch cilia. (F) Particle moves toward metatroch; *3 indicates a metatroch cilium just before its effective stroke. (G, H) During its effective stroke, the same metatroch cilium remains in association with the particle. (I) The particle (black arrow) is transported into the food groove concomitant with the recovery stroke of the metatrochal cilium.

lower than 1, which would indicate that the particle was traveling faster than the cilium. This is not expected for a particle passing through a ciliary band. We suspect that in most cases a measured C/P velocity ratio less than 1 reflects a limit to accuracy of the measurements, but a cell of *Rhodomonas* sp. that moved from the trailing to the leading side of a cilium may have moved by its flagellum, thus traveling faster than the intercepting cilium that propelled it.

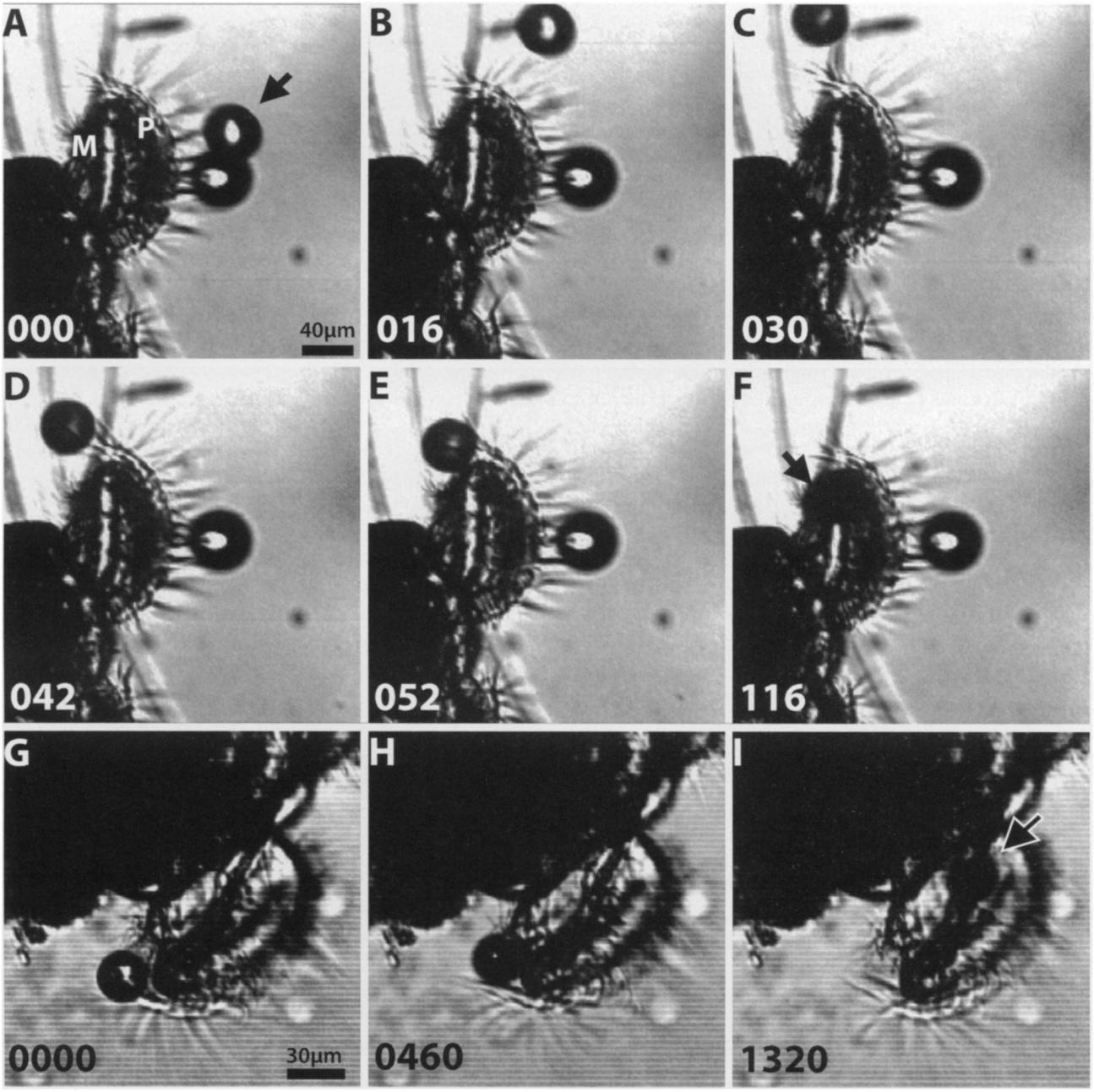

Figure 12. Two events of capture and transport of two large plastic spheres. Ventral view of entire velar lobe (see supplementary video clip #298, http://www.biolbull.org/supplemental/). Labels of ciliary bands in frame A and times as in Fig. 2. (A) Arrow points to incoming sphere 44 μm in diameter. (Another sphere lies on the bottom of the dish in each frame.) (B–D) Captured sphere is moved around the lateral edge of the velum. (E) The sphere is moved to the food groove and spun for 64 ms before (F) it is placed on the food groove and moved toward the mouth at 0.13 μm ms^{-1} (arrow points to sphere). (G) A different larva has already captured a sphere of 27.5 μm in diameter. The sphere was spun near the food groove for at least 460 ms before (H) it was placed on the food groove and (I) transported down the food groove at 0.09 μm ms^{-1} (arrow points to sphere).

In some cases, the cilium that had captured a particle bent and slowed toward the end of its effective stroke as an adjacent cilium passed by (Fig. 11C, D). The recordings show that a cilium appears to be in contact with the captured particle through the later part of the effective stroke and perhaps even in the recovery stroke, but we could not measure C/P velocity ratio in the recovery stroke.

If a ciliary feeding mechanism of veligers is direct interception, what allows the cilia to adhere to particles and not lose them to tangential drag forces as they are swept toward

the food groove? One possibility is mucus, which may or may not be present in the food groove. The cases in which a prototrochal cilium tugged a diatom chain into the food groove while in contact with one part of the chain provide strong evidence for adhesion between cilium and particle (Fig. 2). The particles intercepted and captured by prototrochal cilia in our study included three kinds of algae and plastic spheres. The surface properties of these particles are presumably diverse. Veligers capture an even wider variety of particles. Whatever adhesive material they use must be capable of sticking to diverse materials.

Variation in whether particles were intercepted or passed by prototrochal effective strokes suggests, as a hypothesis, that adhesion varies, perhaps from variation in secretion of mucus. Secretion of mucus in the food groove has been suggested because rejected particles are entangled in a transparent material (Lebour, 1931; Strathmann *et al.*, 1972). Variation in secretion of an adhesive would provide a mechanism for regulating capture rates along the prototrochal band while the cilia continued beating. Previous observations of a variety of opposed-band feeders have demonstrated rejection of particles at the mouth after they had been captured and transported in the food groove (Werner, 1955; Fretter, 1967; Strathmann *et al.*, 1972; Gallager, 1988; Hansen, 1991). Fretter and Montgomery (1968) and Strathmann *et al.* (1972) suggested regulation of rate of capture at the prototrochal and metatrochal bands but did not suggest variable adhesion as a mechanism. Testing for regulation of capture by variation in the adhesiveness of prototrochal cilia awaits further observations.

Direct interception as a feeding mechanism for opposed-band feeders requires stiff prototrochal cilia. With few exceptions, the prototroch cilia are compound, which provides greater stiffness, velocity, beat frequency, and length of the cilia (Harris, 1961; Strathmann *et al.*, 1972; Sleigh, 1984; Gallager, 1988; Emlet and Strathmann, 1994). Are compound cilia necessary for direct interception of particles? The mitraria larva of oweniid annelids feeds with opposed prototrochal and metatrochal bands of simple cilia (Emlet and Strathmann, 1994). Requirements for ciliary stiffness may be less because the cilia are short and velocities low, but the interactions of cilia and particles have not been observed.

The video recordings suggest that the prototrochal recovery stroke helps move particles to the food groove. Indeed, particles intercepted by a prototrochal cilium appear to detach from the cilium during the recovery stroke just as the cilium tip passes over the edge of the velum. Possibly another prototrochal cilium in its effective stroke pushes the particle toward the food groove. Additionally, the current produced from the metatroch effective strokes may move the particle toward the food groove. In some cases, the particle remained within the area of prototrochal recovery strokes for more than 10 ms, detained either without adhesion to cilia or with brief adhesion to a succession of cilia.

Variations in captures show the diversity of possible capture mechanisms. Captures ranged from multiple interceptions by successive effective strokes to no interceptions by prototrochal effective strokes. Particles missed by prototrochal cilia were sometimes retained by recovery strokes or metatrochal cilia.

It is remarkable that 20-, 30-, and 40-μm spheres can be caught and moved along the narrow food groove of these veligers. The larva did not ingest these large spheres, however. Other larvae that capture particles between prototroch and metatroch clear large particles at low rates. Clearance rates are low for particles above about 10 to 20 μm, depending on the size of the ciliary bands and food groove (Hansen, 1991, 1993; Riisgård *et al.*, 2000). The low clearance rates reported for large particles and mechanical considerations had led us to suppose that the width of the food groove set a low upper limit to the size of particles captured and transported, but size ingested appears to be more limiting than the size that can be captured.

In contrast to most previous studies of this feeding mechanism, we observed captures of particles along the prototrochal cilia almost to their tips. Strathmann *et al.* (1972) observed such captures along the much shorter cilia of a rotifer, but Strathmann and Leise (1979) and Gallager (1988) never observed captures near the tip of a prototrochal cilium. Possibly particle capture varies, with different veligers relying on different mechanisms for feeding or adhesion along the length of cilia. Alternatively, each veliger may employ several different mechanisms. In addition to interceptions of particles almost to the tips of cilia, we saw captures with no discernible adhesion to prototrochal cilia in their effective strokes, and these were particles that passed more proximally, close to the recovery strokes. The capture of particles that had been passed by cilia in their effective strokes, together with some long residence times in the zone of recovery strokes, suggests that captures occur by mechanisms in addition to direct interception. Capture between opposed bands of cilia by more than one mechanism could aid feeding on the vast array of prospective palatable particles in marine systems and provide a means to vary clearance rates. It could also explain different observations and inferences by different authors.

Several authors have noted a possible role of the oscillating currents and shear experienced by particles passing through a ciliary band (Jørgensen, 1981; Gallager, 1988; Mayer, 2000). These suggestions have not accounted for clearance rates but do point to steep velocity gradients and fluctuating velocities during cycles of ciliary beat. Effects of shear and oscillation were beyond the scope of our study but might have played a role in observed aspects of particle capture that were not discernibly by direct interception, such as the retention of particles in the vicinity of recovery strokes in some captures.

As noted in the introduction, a variety of planktonic and benthic animals share similar arrangements of opposed

bands of cilia. The evidence of adhesion between prototrochal cilium and captured particle may have implications for other ciliary feeding mechanisms as well. Mussels capture particles with the effective strokes of laterofrontal cilia that beat against the current created by the lateral cilia of the gill filaments, but observations have not demonstrated presence or absence of adhesion between cilium and particle (Riisgård *et al.*, 1996; Silverman *et al.*, 1999, 2000). Captures by direct interception by cilia may occur in diverse animals with a variety of arrangements of cilia.

Acknowledgments

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